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Short sequence-paper

Molecular cloning of cDNAs encoding α1, α2, and β subunits of rat brain platelet-activating factor acetylhydrolase [1]

Machiko Watanabe, Junken Aoki, Hiroshi Manya, Hiroyuki Arai *, Keizo Inoue

Graduate School of Pharmaceutical Sciences, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113, Japan

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Abstract

Brain intracellular platelet-activating factor acetylhydrolase (PAF-AH(Ib)) is a tertiary G-protein-complex-like heterotrimeric enzyme which is composed of α1, α2, and β subunits and is implicated in stages of brain development such as the formation of the brain cortex. We have isolated and sequenced cDNA clones encoding these three subunits of rat brain PAF-AH(Ib). The amino acid sequences of brain PAF-AH has shown an extremely high homology among mammalian species. The tissue distribution of the three subunits was examined by Northern blot analysis. Although the mRNAs were detected in various organs, the ratio of the level of mRNA expression for the three subunits differed among rat tissues, raising the possibility that isoform(s) other than the heterotrimeric isoform exist in certain tissues. © 1998 Published by Elsevier Science B.V.

Keywords: Platelet-activating factor; PAF; PAF acetylhydrolase; α1 Subunit; α2 Subunit; β Subunit

Bovine brain platelet-activating factor acetylhydrolase (PAF-AH(Ib)) is a heterotrimeric enzyme consisting of α1, α2, and β subunits [1]. The catalytic activity of PAF-AH resides in the α1 and α2 subunits, both of which have shown an approximately 60% amino acid homology with each other [2,3]. The β subunit belongs to the family of WD 40 repeat-containing polypeptides that are typically found as subunits of multimeric protein complexes and may mediate protein–protein interactions. Surprisingly, the β subunit has been identified as a product of the *LIS-1* gene, a causative gene for Miller-Dieker lissencephaly which is a brain malformation manifested by a smooth cerebral surface and abnormal neuronal migration [4]. From these observations, it appears that brain PAF-AH plays an important role in brain development, especially in the process of neural cell migration. Because primary cultured neurons prepared from rat brain are often used for in vitro assays of neuronal cell migration [5], it should be useful to isolate the subunit cDNAs for rat brain PAF-AH. In this study, we have isolated and sequenced cDNA clones encoding the α1, α2, and β subunits of rat brain intracellular PAF-AH. Expression of the three subunits in rat tissues were also examined by Northern blot analysis.

Because preliminary experiments have demon-

* Corresponding author. Fax: 81 3 3818 3173; E-mail: harai@mol.f.u-tokyo.ac.jp

[1] The nucleotide sequence data reported in this paper have been submitted to the GenBank database under the accession number AF016047 for α1, AF016048 for α2 and AF016049 for β, respectively.

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strated that rat brain cytosol exhibits a significant level of PAF-AH activity, we isolated the cDNA clones for each subunit from the rat brain cDNA library. From the rat brain (Wistar, female, 7 weeks) poly(A)$^+$ RNA was isolated and a cDNA library was prepared using the Super Script Lamda System (GIBCO BRL). The library was screened with the cDNA fragments of each subunit from bovine PAF-AH(Ib) as described [6]. DNA fragments corresponding to the 5′-coding region (nucleotide number 1–331 of α1, 1–422 of α2 and 1–335 of β) were used as DNA probes. cDNA clones that hybridized to the bovine PAF-AH α1, α2, and β subunits were obtained from 1.3×10^5 plaques of the rat brain cDNA library. Fig. 1. summarizes the series of the overlapping clones obtained in this study. These cDNA clones were digested with *Sal*I and *Not*I restriction enzymes and the insert cDNAs were subcloned into pBScriptSK. The nucleotide sequences analysis of these cDNA inserts (λ29-2, λ30-4, λ30-5 and λ45-3 clones) revealed the presence of open reading frames that cover the entire coding region of each subunit. Fig. 2 shows the nucleotide and deduced amino acid sequences of the α1, α2, and β subunits of rat brain PAF-AH. The complete nucleotide sequences of each subunit of the rat PAF-AH(Ib) cDNA have been submitted to data bases where they are available under the accession number AF016047 for α1, AF016048 for α2 and AF016049 for β subunits, respectively.

The λ29-2 clone containing α1 cDNA in its insert is composed of a total of 865 nucleotides and encodes 232 amino acid residues (Fig. 2(a)). The λ30-4 clone containing α2 cDNA in its insert is composed of a total of 1306 nucleotides and encodes 229 amino acid residues (Fig. 2(b)). Expression of the recombinant rat α1 and α2 proteins in *E. coli*, respectively, resulted in PAF-AH activity, confirming that these clones encode catalytic subunits of rat brain PAF-AH (data not shown). The λ45-3 clone containing βcDNA in its insert is composed of a total of 1872 nucleotides and encodes 410 amino acids residue (Fig. 2(c)). The 32 adenine residues at the 3′-end are probably the poly(A) tail, since a potential

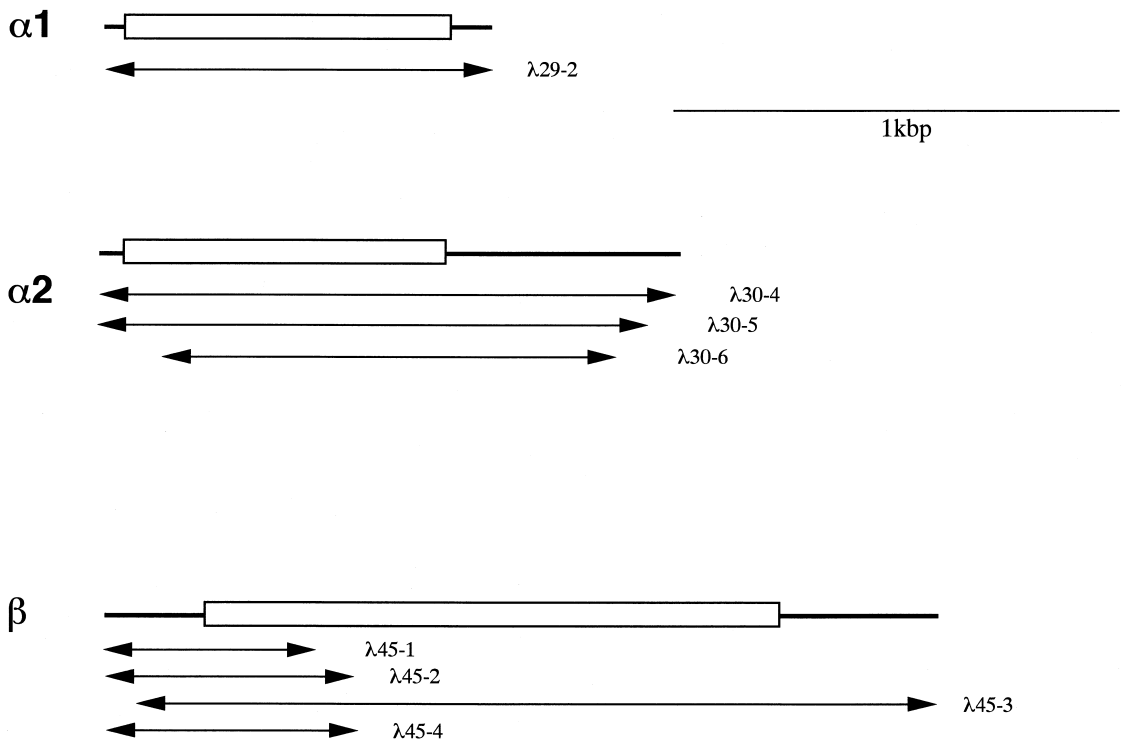

Fig. 1. cDNA clones for α1, α2 and β subunits obtained from the λgt22 rat brain library. The open boxes indicate the open reading frames, solid lines indicate the 5′ and 3′ non-coding regions, and arrows indicate each clone obtained.

polyadenylation signal AATAAA is present only slightly upstream of potential poly(A) sequences (nucleotide 1820–1825) (Fig. 2(c)).

The deduced amino acid sequences of each subunit were compared with those from other species. Amino acid sequences of the rat β subunit were identical to those from the mouse [7] and bovine [4], and only one amino acid substitution was observed between the rat and human β subunits [8] (Fig. 3(c)). Similarly, only one amino acid substitution was observed between rat α2 subunit and both the bovine and human α2 subunits [3,9] (Fig. 3(b)). The amino acid sequence identity of the α1 subunits between the rat and other species is lower than those of the α2 and β subunits. (Fig. 3(a)). The rat α1 subunit, however, still showed a 96.1% amino acid identity with both the bovine and human α1 subunits (Fig. 3(a)).

Expression of PAF-AH(I) in the adult rat and primary cultured cells. Expression of mRNA for each PAF-AH subunit was examined by Northern blot analysis employing the cDNA corresponding to coding region of each subunit as a probe (Fig. 4(a)). The mRNAs (2 μg each lane) examined were derived from the heart, brain, spleen, lung, liver, skeletal muscle, kidney, and testis (a rat multiple tissue Northern blot from CLONTECH). The filter was hybridized to the radiolabelled cDNA fragments in a rapid hybridization buffer (Amersham) at 65°C, and final washes were in 0.1 × SSC, 0.1% SDS at 65°C. The radioactivity was visualized by autoradiography.

(a)

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GGAGGGAGGGGCGCTAAATTGTCTCTGGTCCCTGTCGGCTTTTCAGGAGCTGCGGTGCTGAGGATGAGCGGGGAAGGGGAGAACCCGGCCAGCAAGCCCACGCCTGTGCAGGACGTGCAG 120
                                                    M  S  G  E  G  E  N  P  A  S  K  P  T  P  V  Q  D  V  Q   19

GGCGACGGACGCTGGATGTCTCTGCACCATCGATTTGTGGCAGACAGCAAAGACAAGGAACCCGAAGTCGTCTTTATCGGGGACTCCTTGGTTCAGCTAATGCATCAGTGTGAGATCTGG 240
G  D  G  R  W  M  S  L  H  H  R  F  V  A  D  S  K  D  K  E  P  E  V  V  F  I  G  D  S  L  V  Q  L  M  H  Q  C  E  I  W   59

CGGGAGCTCTTCTCACCTCTGCATGCACTTAACTTTGGCATTGGCGGTGACAGCACACAGCATGTACTCTGGCGGCTTGAGAATGGGGAGCTGGAGCACATCCGGCCCAAGATTGTGGTG 360
R  E  L  F  S  P  L  H  A  L  N  F  G  I  G  G  D  S  T  Q  H  V  L  W  R  L  E  N  G  E  L  E  H  I  R  P  K  I  V  V   99

GTCTGGGTGGGCACCAACAACCACAGCCACACAGCAGAACAAGTGACAGGTGGCATCAAAGCCATTGTACAACTGGTGAACAAGCTGCAGCCCCAGGCCCGGGTGGTTGTGCTGGGCCTG 480
V  W  V  G  T  N  N  H  S  H  T  A  E  Q  V  T  G  G  I  K  A  I  V  Q  L  V  N  K  L  Q  P  Q  A  R  V  V  V  L  G  L  139

CTTCCAAGAGGCCAGCACCCCAACCCACTTCGAGAGAAAAACCGACAGGTGAACGAGCTGGTTCGGGCAGCACTAGCTGGCTACCCCCGAGCGCACTTCTTAGATGCAGACCCTGGCTTT 600
L  P  R  G  Q  H  P  N  P  L  R  E  K  N  R  Q  V  N  E  L  V  R  A  A  L  A  G  Y  P  R  A  H  F  L  D  A  D  P  G  F  179

GTGCACTCGGACGGTACCATAAGTCACCATGACATGTATGACTACCTGCACCTGAGCCGCCTGGGGTACACACCTGTCTGCCGGGCCCTGCACTCCTTGCTGCTTCGTCTCCTGGCCCAA 720
V  H  S  D  G  T  I  S  H  H  D  M  Y  D  Y  L  H  L  S  R  L  G  Y  T  P  V  C  R  A  L  H  S  L  L  L  R  L  L  A  Q  219

GATCAGGGCCAAGGCATCCCTCTGCCAGAGACAGCACCCTAAGCATCTTTCCTTCCTTCCCTTGACAATAAATTCTCCTCTTCCACCTAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA 840
D  Q  G  Q  G  I  P  L  P  E  T  A  P  *  232

AAAAAAAAAAAAAAAAAAAAAAA 865
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(b)

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AACTGGACGAATTTTTTTTTTTTTTTTTTTGGTTCTTTTTTTCGGAGCTGGGGACCGAACCCAGGGGCCTTGCGCTTCCTAGGTGTAGAATGAGCCAAGGAGACTCAAACCCAGCAGCT 118
                                                                M  S  Q  G  D  S  N  P  A  A   10

ATTCCACATGCAGCAGAAGATATTCAAGGAGATGACAGGTGGATGTCTCAGCACAATAGGTTTGTTCTGGACTGCAAAGACAAAGAGCCGGACGTGCTGTTTGTGGGAGACTCCATGGTA 238
I  P  H  A  A  E  D  I  Q  G  D  D  R  W  M  S  Q  H  N  R  F  V  L  D  C  K  D  K  E  P  D  V  L  F  V  G  D  S  M  V   50

CAGTTGATGCAGCAGTACGAGATATGGCGGGAGCTGTTTTCCCCACTTCATGCTCTTAATTTTGGAATTGGGGGAGACACAACACGACATGTTTTATGGAGACTAAAGAACGGAGAGCTG 358
Q  L  M  Q  Q  Y  E  I  W  R  E  L  F  S  P  L  H  A  L  N  F  G  I  G  G  D  T  T  R  H  V  L  W  R  L  K  N  G  E  L   90

GAGAACATTAAGCCTAAGGTCATTGTTGTCTGGGTAGGAACAAACAACCATGAAAATACAGCAGAAGAAGTAGCAGGTGGAATTGAGGCTATCGTACAGCTTATAAACACGAGGCAGCCA 478
E  N  I  K  P  K  V  I  V  V  W  V  G  T  N  N  H  E  N  T  A  E  E  V  A  G  G  I  E  A  I  V  Q  L  I  N  T  R  Q  P  130

CAGGCCAAGATCATTGTACTGGGTCTGTTACCTCGAGGTGAGAAGCCCAACCCTTTAAGACAAAAGAATGCCAAGGTGAACCAGCTTCTCAAGGTTTCCCTGCCGAAGCTTGCCAATGTG 598
Q  A  K  I  I  V  L  G  L  L  P  R  G  E  K  P  N  P  L  R  Q  K  N  A  K  V  N  Q  L  L  K  V  S  L  P  K  L  A  N  V  170

CAGCTCCTGGATATAGATGGGGGCTTCGTGCACTCGGACGGTGCCATCTCCTGCCACGACATGTTTGATTTTCTTCATCTCACAGGAGGGGGCTATGCAAAGATCTGCAAACCCCTCCAT 718
Q  L  L  D  I  D  G  G  F  V  H  S  D  G  A  I  S  C  H  D  M  F  D  F  L  H  L  T  G  G  G  Y  A  K  I  C  K  P  L  H  210

GAACTGATCATGCAGTTGCTGGAGGAAACACCAGAGGAGAAGCAGACCACCATTGCTTGATGACTGGCCCATCAGTGTTAACAGCATCCCAGCTTCCTCAGATCAGTTAGACCACTGGCA 838
E  L  I  M  Q  L  L  E  E  T  P  E  E  K  Q  T  T  I  A  *  229

CTACAGAATCCTTTCTTAAGGCACTTTGCATTGTACAATGTTCCTGGGTGCTTGTATGTTGAAGGGGAGGAGGGATTTAATTTGGTCCTGTATATAAAGGTTTGTTTGAAGAGAGGAGAA 958
AAATTAGCCAAGGAAGATTCATTTGAAACCAGAAGGGGACTTTTTAGGTGTTTATGTCACACTTGTAGAATTATCACTGTTTTTCCTAGGACAACATCAAGCAAGAGTACAATATGAAGA 1078
TTCTTTTCTTGGCCCCTTTTTTCTATGGATTATGTCATGTGTGATAATTATCACAGCTACTTGGCTTTAAACTTATTTTTGGTCCAGTTTTAAGGTTCATGAGTTAGTATGTTGTGTTGT 1198
GTTGTTTGATTCATGTATGCTCGGTAATTTTATTAACATATAGCATCAGAATAAACATGCTTGTCACTCCAGTATGCGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA 1306
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Fig. 2.

Fig. 2. cDNA and amino acid sequences of α1 (a), α2 (b), and β (c) subunits of the rat brain PAF-AH(Ib). The first and second lines indicate the nucleotide and the deduced amino acid sequence, respectively. Nucleotide and amino acid positions are shown at the right. The start Met codon, the stop codon, and the polyadenylation signal are underlined.

Transcripts encoding the β subunit consisting of 7.1 and 2.2 kb messages were seen in all tissues, with the highest expression in the brain. On the other hand, both the α1 and α2 mRNAs were not detected in skeletal muscle, and heart did not express α1 mRNA (Fig. 4(a)). The expression of the α1 subunit in adult rat tissues appears to be fairly low because a long exposure was required to detect any α1 mRNA. An α1 probe detected a single band of 1.16 kb. In contrast, a Northern blot analysis of α2 gene expression demonstrated a transcript of 3.3 kb in most tissues in which the gene was expressed, with additional hybridization to mRNAs of 4.4, 2.2 and 1.35 kb in some tissues. Expression of α1 and α2 in rat primary neuron, astroglia, fibroblast and microglia was also examined. Total cellular RNA was isolated from each culture [10] by the guanidium thiocyanate/CsCl method, the RNA sample (10 μg) were electrophoresed, transferred onto a HybondN+ membrane (Amersham) and the filter was hybridized with α1 and α2 probes as described above. The α2 transcripts of 4.4, 3.3, 2.2, and 1.35 kb were detected in primary neuron, astroglia and microglia (Fig. 4(b)) as in rat tissues (Fig. 4(a)) and the α2 transcripts of 4.4 and 2.2 kb were detected in fibroblast. Ubiquitous expression of β transcript was also observed (Fig. 4(b)). On the contrary, the α1 transcript was highly expressed only in primary neuron and very weak expression of α1 was observed in astroglia (Fig. 4(b)).

The α1 and α2 subunits are ~60% identical with each other. We have previously shown that both the α1 and α2 subunits form a homodimer (α1/α1 and α2/α2) when expressed individually in *E. coli* [3]. These results raise the possibility that such homodimers or a heterotrimer containing the β subunit

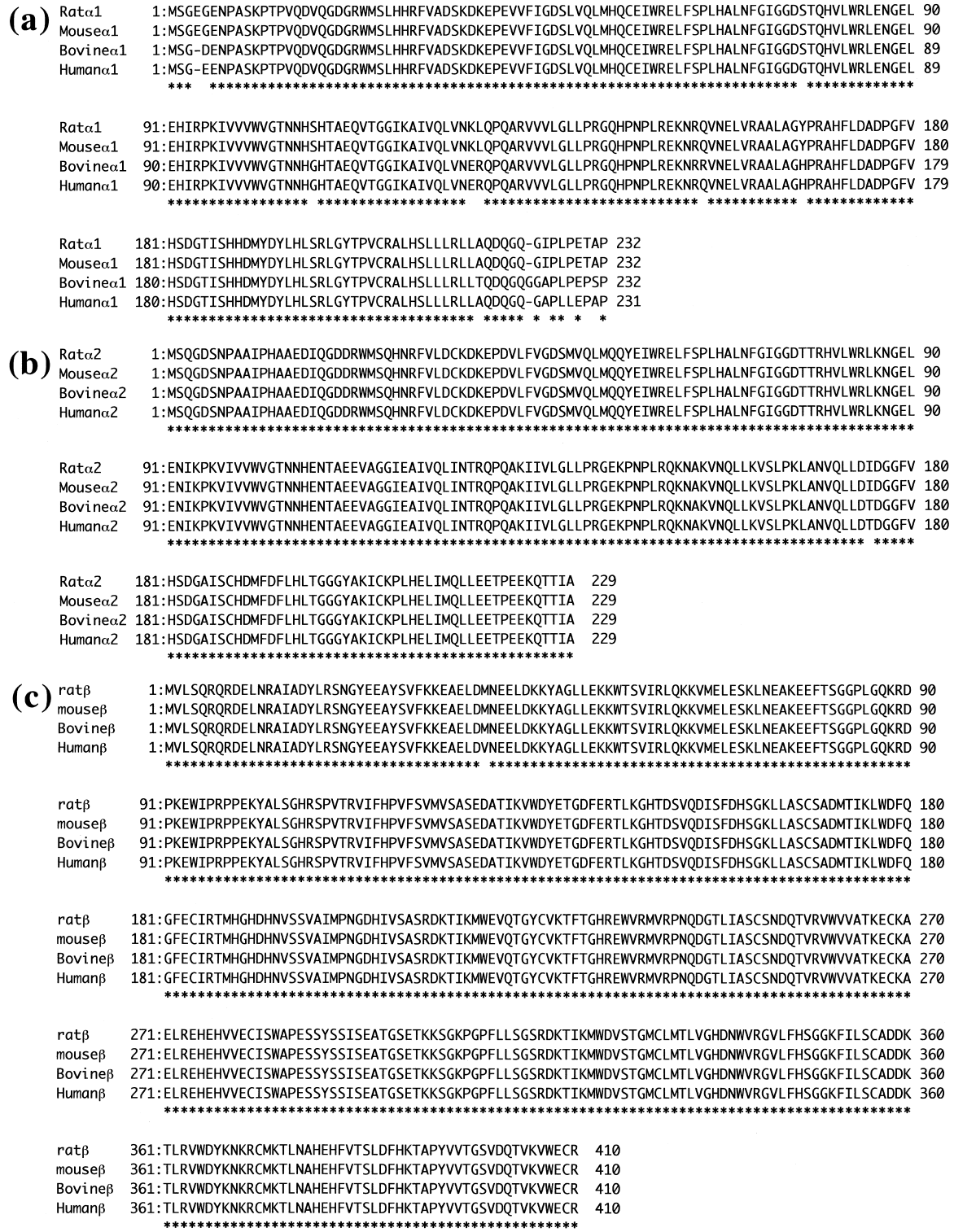
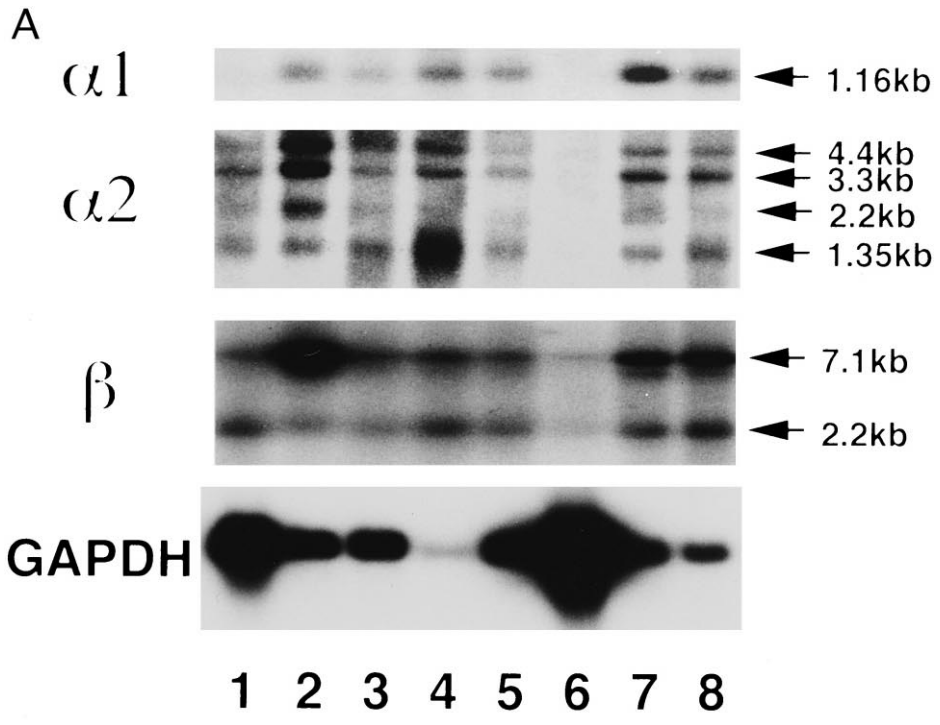
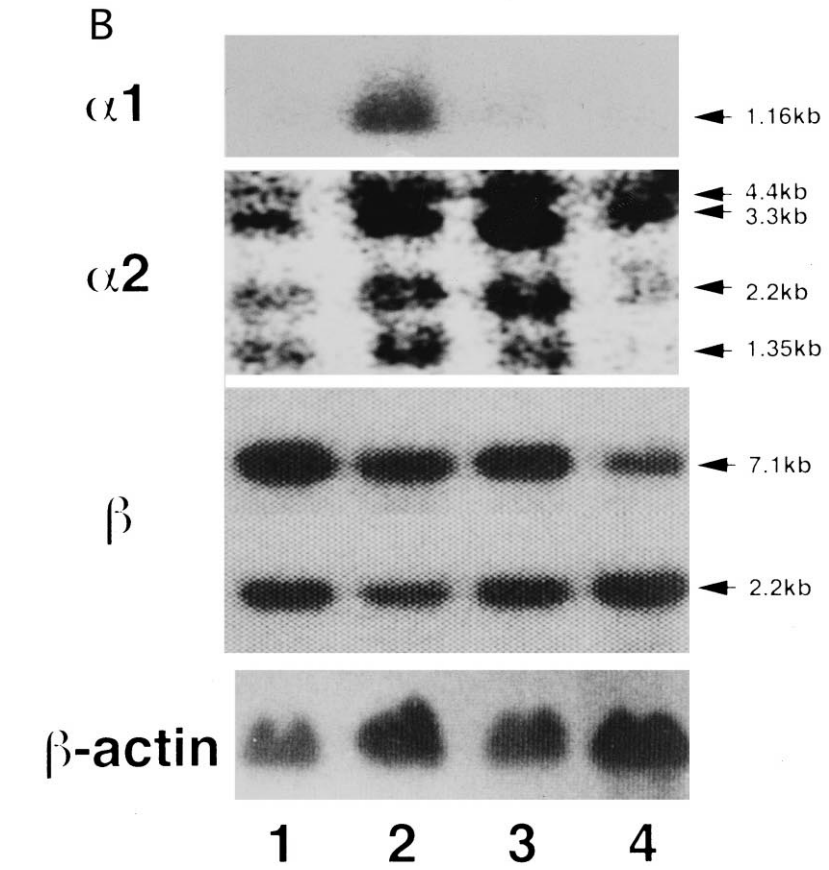


Fig. 3. Amino acid sequence comparison of α1 (a), α2 (b), and β (c) protein from various species. Identical amino acid residues are indicated by an asterisk.


A
α1
1.16kb
α2
4.4kb
3.3kb
2.2kb
1.35kb
β
7.1kb
2.2kb
GAPDH
1 2 3 4 5 6 7 8


B
α1
1.16kb
α2
4.4kb
3.3kb
2.2kb
1.35kb
β
7.1kb
2.2kb
β-actin
1 2 3 4

(α1/α1/β and α2/α2/β) may exist in rat tissues in addition to the classical isoform Ib (α1/α2/β heterotrimer) [1]. It seems likely that rat primary neuron express classical α1/α2/β heterotrimer, whereas astroglia, fibroblast and microglia express α2/α2/β heterotrimer, which has not been detected yet, since α1 expression was not observed in these cells (Fig. 4(b)).

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Fig. 4. Northern blot analysis of PAF-AH in various adult rat tissues (a) and in promary cultured cells (b). (a) Two μg of polyA$^+$ RNA on a nylon membrane (Rat Multiple Tissue Northern, CLONTECH) were hybridized with four probes specific to α1, α2, β of rat PAF-AH(Ib), and GAPDH. Lane 1. heart, 2. brain, 3. spleen, 4. lung, 5. liver, 6. skeletal muscle, 7. kidney, 8. testis. (b) Ten μg of total RNA on a nylon membrane were hybridized with four probes specific to α1, α2, β of rat PAF-AH(Ib), and β-actin. Lane 1. microglia, 2. neuron, 3. astroglia, 4. fibroblast. Molecular weight of each transcript calculated from molecular weight standard was shown at the right.

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